bio-protocol Preprint

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Lectin staining

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An abbreviated version of this protocol was published in eLIFE in Mar 2019

A conserved major facilitator superfamily member orchestrates a subset of O-glycosylation to aid macrophage tissue invasion

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Detailed protocol

1. Collect embryos on apple juice plates for 6 to 8 hours at 29°C. After collection, either fix immediately or store at +4°C, maximum 2 days.
2. Prepare Fixing Solution in Scintillation Vial (1ml PBS, 1ml 10% methanol-free Formaldehyde, 8ml Heptane).
3. Incubate collected embryos with 50% Chlorox for 5 min.
4. Collect bleached embryos on a mesh and wash with deionized water.
5. Transfer Embryos to fixing solution.
6. Fix Embryos for 20 minutes at room temperature with slight shaking.
7. Remove all Formaldehyde from the bottom of the scintillation vial (important step, be precise and careful).
8. Add 80% Ethanol to the mix and shake for 2 minutes.
9. Let embryos sink to bottom for approx. 5-10 minutes.
10. Collect embryos and wash first with 75%, then with 30% Ethanol.
11. Remove Ethanol and add PBS to the embryos
12. Continue with staining or store at 4°C for up to 4 weeks.
13. Block embryos with BBT (0.1M PBS + 0.1% TritonX-100 +0.1% BSA) for 2 hours at room temperature.
14. Dilute the FITC-labeled lectin 1:25.
15. Remove BBT from embryos and add appropriate amount of the diluted lectin to fixed embryos.
16. Incubate overnight at room temperature with slow rotation, protected from light.
17. After incubation, wash embryos in BBT for 2 hours at room temperature with slow rotation. Exchange BBT every 30 min. Protect from light all the time.
18. Remove all BBT and add Vectashield.
19. Incubate overnight at 4°C, protected from light.
20. Mount the embryos on a slide and image on a confocal microscope.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Valoskova, K. (2020). Lectin staining. Bio-protocol Preprint. bio-protocol.org/prep392.
2. Valoskova, K., Biebl, J., Roblek, M., Emtenani, S., Gyoergy, A., Misova, M., Ratheesh, A., Reis-Rodrigues, P., Shkarina, K., Larsen, I. S. B., Vakhrushev, S. Y., Clausen, H. and Siekhaus, D. E.(2019). A conserved major facilitator superfamily member orchestrates a subset of O-glycosylation to aid macrophage tissue invasion. eLIFE. DOI: 10.7554/eLife.41801